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¹ Air Force Research Laboratory Human Effectiveness Directorate Biosciences and Performance Division Biobehavior, Bioassessment, & Biosurveillance I 2485 Gillingham Dr. Brooks City-Base, TX 78235-5105	² General Dynamics Advanced Information Services 5200 Springfield Pike Branch Dayton, OH 45431	N/A	
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13. SUPPLEMENTARY NOTES

The purpose of this study was to investigate the ability of whey-protein and leucine supplementation to enhance physical and cognitive performance and body composition. Thirty moderately fit participants completed a modified Air Force fitness test, a computer-based cognition test, and a dual-energy X-ray-absorptiometry scan for body composition before and after supplementing their daily diet for 8 wk with either 19.7 g of whey protein and 6.2 g leucine (WPL) or a calorie-equivalent placebo (P). Bench-press performance increased significantly from Week 1 to Week 8 in the WPL group, whereas the increase in the P group was not significant. Push-up performance increased significantly for WPL, and P showed a nonsignificant increase. Total mass, fat-free mass, and lean body mass all increased significantly in the WPL group but showed no change in the P group. No differences were observed within or between groups for crunches, chin-ups, 3-mile-run time, or cognition. The authors conclude that supplementing with whey protein and leucine may provide an advantage to people whose performance benefits from increased upper body strength and/or lean body mass.

15. SUBJECT TERMS

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The Influence of 8 Weeks of Whey-Protein and Leucine Supplementation on Physical and Cognitive Performance

Thomas B. Walker, Jessica Smith, Monica Herrera, Breck Lebegue, Andrea Pinchak, and Joseph Fischer

The purpose of this study was to investigate the ability of whey-protein and leucine supplementation to enhance physical and cognitive performance and body composition. Thirty moderately fit participants completed a modified Air Force fitness test, a computer-based cognition test, and a dual-energy X-ray-absorptiometry scan for body composition before and after supplementing their daily diet for 8 wk with either 19.7 g of whey protein and 6.2 g leucine (WPL) or a calorie-equivalent placebo (P). Bench-press performance increased significantly from Week 1 to Week 8 in the WPL group, whereas the increase in the P group was not significant. Push-up performance increased significantly for WPL, and P showed a nonsignificant increase. Total mass, fat-free mass, and lean body mass all increased significantly in the WPL group but showed no change in the P group. No differences were observed within or between groups for crunches, chin-ups, 3-mile-run time, or cognition. The authors conclude that supplementing with whey protein and leucine may provide an advantage to people whose performance benefits from increased upper body strength and/or lean body mass.

Keywords: nutritional supplementation, exercise, body composition, ergogenics

Military personnel routinely face rigorous environments, schedules counter to normal circadian physiology, and physically and mentally demanding tasks. Mission completion is so important that some military personnel take medication or dietary supplements of unknown utility and safety to accomplish it. In a 2006 survey of U.S. Air Force (USAF) members, 69% of respondents admitted to either currently using or previously using dietary supplements (Greenwood & Oria, 2008). However, only 19% had been provided any official guidance or education on the efficacy and safety of the supplements they were using. These numbers are very similar to previous findings of supplement use in U.S. Army soldiers (Bovill, Tharion, & Lieberman, 2003). It would be advantageous to identify the nutritional supplements that could safely and effectively increase military-relevant performance. The dietary combination of whey protein and leucine has promise to be such a supplement.

Supplementation with leucine (Crowe, Weatherson, & Bowden, 2006) and whey protein (Burke et al., 2001) has been shown to improve single-bout exercise performance and chronically increase nitrogen balance and promote anabolism, thereby resulting in greater

physical strength. Crowe et al. observed a 14% increase in exercise time to exhaustion and a 12% increase in upper body power in rowers who were supplemented for 6 weeks with 45 mg · kg⁻¹ · day⁻¹ of L-leucine. Work by Koopman et al. (2005) has suggested that the combination of whey protein with leucine may be more powerful than either supplement alone to increase whole-body net protein balance. Similarly, Coburn et al. (2006) recently reported that the combination of whey protein with leucine elicited greater strength gains (30%) after 8 weeks of supplementation and unilateral leg-extension resistance training than did a carbohydrate placebo (22%).

Protein and branched-chain amino acids (BCAA) may also improve cognitive performance during fatigue. Blomstrand, Hassmén, Eckblom, and Newsholme (1991, 1997) and Hassmén, Blomstrand, Eckblom, and Newsholme (1994) observed that participants supplemented with BCAAs scored better on both mood levels and cognitive tasks after exercise. However, other studies have not supported this thesis (Cheuvront et al., 2004).

Improvements in strength and cognition likely translate directly into increased operational capability for military personnel, particularly our special operators. The purpose of this study was to investigate the ability of 8 weeks of whey-protein and leucine supplementation to enhance physical and cognitive performance and body composition.

Walker, Smith, Herrera, Lebegue, and Pinchak are with the Air Force Research Laboratory, Brooks Air Force Base, San Antonio, TX. Fischer is with General Dynamics Advanced Information Services in San Antonio.

Methods

Volunteers

After the protocol was approved by the Wright-Patterson institutional review board, 35 volunteers signed an informed-consent document and completed a medical screening before participating. Thirty-three participants completed the study, 30 men and 3 women. The study was open to both sexes, but because only 3 female participants completed the study, and because of the inherent difficulty in comparing macronutrient responses between sexes, results for the 3 female participants were removed for this report. The mean age of the remaining 30 male participants was 26.9 ± years, and 24 of the 30 were USAF personnel. All participants met the following inclusion criteria: met the American College of Sports Medicine (2000) definition of low risk, had performed exercise at least three times per week for the past 3 months, and had not used any nutritional supplements for at least 30 days before the study start.

Procedures

On acceptance into the study, participants completed a presupplement session consisting of a dual-energy X-ray-absorptiometry body-composition scan (GE Healthcare, Chalfont St. Giles, UK), a blood draw, and approximately 1 hr of cognitive test-battery training.

Participants underwent a presupplement testing session 2 days after their training. In the session they completed a one-repetition-maximum (1-RM) bench press and maximum chin-up, push-up, and crunch repetitions completed within 1 min. They were given a 3-min break between exercises. After the crunches, participants received a 5-min rest before beginning a timed 3-mile (4.8 km) run. They were required to complete 12 laps on the track in as short a time as possible. They were also asked to sprint as fast as possible for the last 40 yd (36.6 m). This physical testing protocol was a modification of the standard Air Force Physical Fitness Test, combined with elements of the Air Force Special Operations Physical Ability Stamina Test. After the run, participants took a 10- to 15-min break before beginning the cognitive testing. The computer-based cognitive testing took approximately 15-20 min and included the following elements of the Automated Neuropsychological Assessment Metric (Jones, Loe, Krach, Rager, & Jones, 2008): the Continuous Performance Task, the Sternberg Memory Task, and the Stanford Sleepiness Scale. During all testing, participants were allowed water ad libitum but were not allowed to consume anything else.

After the presupplement testing, participants were assigned to either the protein group or the placebo group in a randomized double-blind manner. At final count there were 18 men in the protein group and 12 in the placebo group. (The imbalance between groups was the result of randomization with the initial goal of 40 participants, 2 participants who started but did not complete the protocol, and the exclusion of the 3 female participants' data

from the final results.) Participants consumed the protein or placebo daily for 8 weeks. Each packet of the protein treatment contained 112 kcal, including 19.7 g of whey protein and 6.2 g leucine. Placebo packets contained 112 kcal of carbohydrate with 0.0 g protein. On days they exercised, participants consumed one packet of powder 30-45 min before exercising and the second packet 30-45 min afterward. They were instructed not to consume anything other than the supplement within 1 hr before and after exercise. On nonexercise days, participants consumed both packets in the morning. Throughout the 8-week supplementation period they were required to maintain USAF standards of physical training, meaning that at least 3 days a week they engaged in endurance training (running) along with push-ups and crunches. If participants had been exercising at volumes or intensities above these minimum requirements before the start of the study, they were allowed to continue doing so. If they had not done so, they were instructed to not exceed USAF minimum physical-training guidelines during the study. Each day participants recorded their exercise to include activity, duration, and intensity. On study completion, we categorized the participants into three groups. Those who participated in at least 2 hr of resistance training (RT) per week over and above minimum guidelines were categorized as high-RT, those completing 1-2 hr/ week as medium-RT, and those completing less than 1 hr/week as low-RT. We recorded "packet compliance" by calculating the percent of required packets that were actually consumed by each participant over the duration of the study.

Participants recorded their daily food consumption for 3 days total, once near the beginning of the 8-week period and again near the end of the 8 weeks. The food logs were used primarily to ensure that they had not made substantial changes (e.g., \pm 500 kcal/day) in their dietary habits during the 8 weeks of the study but also to compare caloric intake between groups.

At the end of the first 4 weeks participants returned to the laboratory. Compliance was measured and a medical screening accomplished, but no testing was conducted at that time. After the final 4 weeks of consuming the supplement or placebo, participants completed postsupplement training and testing. These procedures were identical to the presupplement training and testing procedures.

Data Analysis

Initially, a repeated-measures analysis of variance (ANOVA) with one within-participant factor (week) and one between-participants factor (treatment group) was performed on each outcome measure. Two covariates (level of resistance exercise during the study: high, medium, low; and packet compliance: % of packets taken) were included in the analysis to adjust for potential bias within the groups. After reviewing the outcomes of these initial analyses (details to be discussed in the Results section), we reanalyzed the data using ANOVAs with the covariates removed and performed additional Student's

paired t tests for each group, separately, to determine whether there were significant changes from Week 1 to Week 8. This deviates from the classical stance that post hoc tests should not be performed unless a significant interaction is found. Given the sample sizes available in this study, we were concerned about the power of the interaction test. For example, to detect a relatively large standardized effect size (e.g., ES = 1), the power of the interaction test is only about .75. On the other hand, the paired t tests that we used when testing for Week 1 to Week 8 changes have a power of >.9 for detecting an ES =1. Although this approach made us more vulnerable to committing a Type I error, we felt that it would be better to err on the liberal side when it came to recommending or not recommending the use of the supplements in the event that even a small supplement effect may give athletes, or war fighters, an advantage. Finally, viewing the data from a different perspective, we calculated for each outcome measure the percentage of participants in each group who showed at least a 5% improvement and compared these percentages using a chi-square test. The purpose of this approach was to test the hypothesis that if the supplement proved not to be beneficial to all participants, it might at least show large beneficial effects on a greater subset of the participants than would be found in the placebo group. All testing was performed at the .05 level of significance.

Results

Influence of Uncontrolled Factors

There were two uncontrolled factors (covariates) that we felt might bias the tests of our primary hypothesis that Week 1–8 changes would differ between the protein and placebo groups: the amount of RT that the individuals were routinely performing and compliance in taking the supplement or placebo packets. In the protein group, 4 participants were classified as low-RT, 5 as medium-RT, and 6 as high-RT. Information was not available for the remaining 3 participants. Their packet compliance ranged from 63% to 100%, with only 1 participant below 80%. In the placebo group, 6 were low-RT, 1 was medium-RT, and 3 were high-RT, with information unavailable for 2. Their packet compliance ranged from 77% to 100%, with only 2 participants below 80%.

For each outcome measure of the study, we performed a repeated-measures ANOVA with treatment group as a between-participants factor and week as a within-participant factor, and we included the two covariates described previously. We found no statistical evidence, for any of the outcome measures, that either of the covariates might be biasing our primary tests (i.e., there were no significant Group × Week × Packet Compliance interactions, and no significant Group × Week × RT interactions). Because of these findings, we decided to reanalyze the data, ignoring the covariates. This allowed us to increase the sample size and, consequently, the power for the primary tests of interest. (Recall that there

were 5 participants for whom we did not have covariate information, and our initial tests were therefore based on a reduced number of participants.) The results of the final statistical tests are discussed here and summarized in Tables 1 (physical performance), 2 (body composition), and 3 (cognitive performance). Some data were missing from the final data sets as a result of three cognitive-test computer files being corrupted and non-study-related injuries to 2 participants that limited their ability to complete all the physical posttests. This is reflected in the sample sizes shown in the tables.

Physical Performance

Overall, bench-press performance increased significantly (i.e., significant week main effect). However, despite the fact that there was not a significant group-by-week interaction, visual inspection suggested that most of the improvement occurred in the supplemented (WPL) group. Student's t tests showed a significant 3.5-kg increase (3.9% improvement) from Week 1 to Week 8 in the WPL group, whereas the increase in the placebo group was not significant (1.3 kg for a 1.4% improvement). Furthermore, from the perspective of "large" changes, we found that 55.6% (10 of 18) of the WPL participants showed a 5% or greater improvement compared with only 16.7% (2 of 12) of the placebo participants. These percentages were significantly different, $\chi^2(1 \, df) = 4.54$, p = .033.

Overall push-up performance increased significantly (significant week main effect). Again, even though there was no significant interaction, most of the improvement was in the WPL group, which showed a significant increase of 5.4 push-ups (12.8% improvement), compared with the placebo group, which had a nonsignificant increase of 3.3 push-ups (7.6% improvement). The percentage of participants who showed large improvement (i.e., 5% or more) in the WPL group was somewhat higher than in the placebo group (72.2% vs. 50%), but these two percentages did not differ statistically.

Crunch performance in the WPL group increased by 3.2 crunches (7.2% improvement), compared with a 1.6-crunch increase (3.4% improvement) in the placebo group. Neither of these increases was significant, and they did not differ statistically from each other. In addition, the percentage of participants who showed 5% or greater improvement was comparable for the WPL and placebo groups (55.6% vs. 58.3%, respectively).

For chin-ups there was a significant group main effect. On average, the WPL group only completed about half as many chin-ups as the placebo group at Week 1. (Participants were randomly selected for each group and were not match-paired. Thus it was solely by chance that the groups differed at the initial pretest.) Chin-ups improved by 0.6 (10.1%) and 0.2 (1.7%) repetitions for the WPL and placebo groups, respectively. Although the 10.1% improvement may appear impressive for the WPL group, we point out that even a small change in the WPL group would result in a fairly large percent change because of the low count at Week 1. Neither of

Table 1 Physical Performance, $M \pm SD$, and Test Results

							ANOVA Results	
Variable	Group	n	Week 1	Week 8	Change	Group	Week	Group × Week
Bench press (kg)						F(1, 28) = 0.00 p = 0.946	F(1, 28) = 7.13 p = 0.012	F(1, 28) = 1.47 p = .235
	WPL	18	89.4 ± 24.0	93.0 ± 24.0	$3.5 \pm 5.2*$			
	placebo	12	91.1 ± 15.6	92.4 ± 17.3	1.3 ± 4.4			
Chin-ups						F(1, 27) = 11.04 p = 0.003	F(1, 27) = 1.29 p = 0.266	F(1, 27) = 0.45 p = .508
	WPL	17	5.9 ± 4.7	6.5 ± 4.5	0.6 ± 1.8			
	placebo	12	12.1 ± 5.0	12.2 ± 5.4	0.2 ± 2.1			
Crunches						F(1, 28) = 0.00 p = .993	F(1, 28) = 2.46 p = .128	F(1, 28) = 0.29 p = .597
	WPL	18	44.4 ± 14.3	47.6 ± 14.9	3.2 ± 7.3			
	placebo	12	45.2 ± 10.0	46.8 ± 10.1	1.6 ± 9.5			
Push-ups						F(1, 28) = 0.08 p = .786	F(1, 28) = 11.57 p = .002	F(1, 28) = 0.71 p = .407
	WPL	18	42.2 ± 14.6	47.6 ±15.3	$5.4 \pm 6.8 *$			
	placebo	12	41.9 ±11.4	45.2 ± 9.1	3.2 ± 6.8			
Sprint (s)						F(1, 25) = 1.92 p = .178	F(1, 25) = 3.90 p = .060	F(1, 25) = 0.00 p = .965
	WPL	15	6.7 ± 1.1	6.4 ± 0.8	-0.3 ± 0.7			
	placebo	12	6.2 ± 1.0	5.9 ± 1.2	-0.3 ± 0.7			
3-mile run (min)						F(1, 25) = 0.82 p = .374	F(1, 25) = 1.95 p = .174	F(1, 25) = 0.29 p = .596
	WPL	16	28.2 ± 5.0	27.8 ± 4.2	-0.4 ± 1.4			
	placebo	11	27.1 ± 2.5	26.2 ± 3.3	-0.9 ± 3.3			

Table 2 Body Composition, $M \pm SD$, and Test Results

							ANOVA Results	
Variable	Group	n	Week 1	Week 8	Change	Group	Week	Group × Week
Body weight (kg)				AT		F(1, 28) = 0.81 p = .376	F(1, 28) = 0.08 p = .783	F(1, 28) = 5.87 p = .022
	WPL	18	86.8 ± 16.4	87.8 ± 17.2	$1.0 \pm 1.8*$			
	placebo	12	83.0 ± 7.7	82.3 ± 7.0	-0.8 ± 2.0			
Fat (kg)						F(1, 28) = 5.17 p = .031	F(1, 28) = 0.50 p = .486	F(1, 28) = 2.45 p = .129
	WPL	18	23.1 ± 9.9	23.4 ± 10.1	0.3 ± 1.7			
	placebo	12	15.9 ± 7.8	15.1 ± 7.6	-0.8 ± 1.9			
% fat						F(1, 28) = 7.56 p = .010	F(1, 28) = 1.29 p = .266	F(1, 28) = 1.50 p = .231
	WPL	18	26.8 ± 6.7	26.8 ± 6.5	0.0 ± 1.5			
	placebo	12	19.7 ± 8.4	18.9 ± 8.4	-0.7 ± 1.9			
Fat-free mass (kg)						F(1, 28) = 1.14 p = .295	F(1, 28) = 2.53 p = .123	F(1, 28) = 2.71 p = .111
	WPL	18	63.7 ± 8.4	64.4 ± 8.7	$0.7 \pm 1.2*$			
	placebo	12	67.1 ± 6.6	67.1 ± 6.4	-0.0 ± 0.9			
Lean (kg)						F(1, 28) = 0.87 p = .358	F(1, 28) = 2.41 p = .132	F(1, 28) = 2.31 p = .139
	WPL	18	60.4 ± 7.9	61.0 ± 8.2	$0.7 \pm 1.3*$			
	placebo	12	63.3 ± 6.3	63.3 ± 6.1	0.0 ± 0.9			

Note. WPL = whey protein and leucine. *Significant change from Week 1 to Week 8 (paired t test, p < .05).

Note. WPL = whey protein and leucine. *Significant change from Week 1 to Week 8 (paired t test, p < .05).

Table 3 Cognitive Performance, M ± SD, and Test Results

							ANOVA Results	
Variable	Group	n	Week 1	Week 8	Change	Group	Week	Group × Week
CPT accuracy						F(1, 21) = 0.57 p = .459	F(1, 21) = 1.04 p = .319	F(1, 21) = 0.18 p = .675
	WPL	12	87.9 ± 15.2	88.8 ± 14.1	0.8 ± 7.6			
	placebo	11	90.7 ± 5.0	92.7 ± 4.3	2.0 ± 5.3			
CPT MRTC						F(1, 21) = 0.05 p = .826	F(1, 21) = 4.30 p = .051	F(1, 21) = 0.18 p = .674
	WPL	12	455.5 ± 80.3	424.0 ± 74.1	-31.5 ± 65.8			
	placebo	11	456.0 ± 55.8	435.3 ± 69.1	-20.8 ± 53.8			
Sternberg 2 accuracy						F(1, 23) = 0.49 p = .491	F(1, 23) = 0.15 p = .704	F(1, 23) = 3.94 p = .059
	WPL	14	96.0 ± 4.2	94.4 ± 5.1	-1.6 ± 4.0			
	placebo	11	92.6 ± 8.4	94.9 ± 4.9	2.4 ± 5.9			
Sternberg 2 MRTC						F(1, 23) = 2.57 p = .123	F(1, 23) = 4.53 p = .044	F(1, 23) = 0.15 p = .705
	WPL	14	448.3 ± 80.5	429.4 ± 70.7	-18.8 ± 65.0			
	placebo	11	417.7 ± 29.3	390.6 ± 28.6	-27.1 ±33.3*			
Sternberg 4 accuracy						F(1, 23) = 0.06 p = .808	F(1, 23) = 0.31 p = .585	F(1, 23) = 2.63 p = .119
	WPL	14	94.4 ± 5.2	95.4 ± 4.6	1.0 ± 3.7			
	placebo	11	95.5 ± 4.2	93.4 ± 7.7	-2.0 ± 5.6			
Sternberg 4 MRTC						F(1, 23) = 0.63 p = .434	F(1, 23) = 5.93 p = .023	F(1, 23) = 0.92 p = .347
	WPL	14	506.1 ± 98.7	468.4 ± 88.0	$-37.7 \pm 56.2*$			
	placebo	11	471.7 ± 49.0	455.3 ± 60.3	-16.4 ± 53.7			
Sternberg 6 accuracy						F(1, 23) = 0.25 p = .621	F(1, 23) = 0.32 p = .579	F(1, 23) = 0.03 p = .870
	WPL	14	91.9 ± 11.9	93.3 ± 4.7	1.4 ± 11.5			
	placebo	11	93.4 ± 5.9	94.2 ± 5.8	0.8 ± 5.8			
Sternberg 6 MRTC						F(1, 23) = 0.40 p = .535	F(1, 23) = 12.1 p = .002	F(1, 23) = 0.29 p = .596
	WPL	14	619.7 ± 153.8	553.5 ±117.6	-66.2 ± 124.9			
	placebo	11	603.1 ± 142.4	512.6 ± 66.1	-90.5 ± 92.7 *			

Note. WPL = whey protein and leucine; CPT = cognitive-performance test; MRTC = reaction time.

the changes from Week 1 to Week 8 was significant. The percentage of participants who showed increases of 5% or more were 60.0% and 41.7% for the WPL and placebo groups, respectively, and they did not differ statistically.

For the 3-mile run, there was no significant difference observed between groups or over time (decreases of 0.4 min [1%] vs. 0.9 min [3%] for WPL and placebo, respectively). The percentage of participants in the placebo group who improved by at least 5% was 45.5% (5 of 11), compared with 18.8% (3 of 16) in the WPL group. These percentages also did not differ statistically.

For the sprint, both groups showed a decrease of 0.3 s (4.3% and 4.8% improvement for WPL and placebo, respectively). These changes were not significant and did not differ significantly from each other. See Table 1.

Body Composition

For average body weight, there was a significant groupby-week interaction. Body weight increased significantly by 1.0 kg in the WPL group and decreased nonsignificantly by 0.8 kg in the placebo group. No significant ANOVA results were found for total fat-free mass or lean body mass. However, when we tested for Week 1 to Week 8 changes in each group separately, total fat-free mass and lean body mass both increased significantly (0.7 kg) in the WPL group, and neither changed in the placebo group. For total fat and percent fat, there were significant group main-effect differences, with the WPL group having higher values than the placebo group (these differences, which existed even at Week 1, are clearly not a treatment effect but rather are the "luck of the draw"

^{*}Significant change from Week 1 to Week 8 (paired t test, p < .05).

resulting from our random selection process). There were no significant Week 1 to Week 8 changes in either group. Although the WPL group demonstrated a significant leanmass gain they also gained a nonsignificant 0.3 kg of fat. Therefore, their body composition did not substantially improve. See Table 2. Caloric intake between groups was not significantly different. The WPL group consumed 2.008 ± 441 kcal/day, and the placebo group averaged 2.062 ± 672 kcal/day. Outside of the supplementation, the WPL group consumed 90.9 ± 30.9 g of protein per day and the placebo group consumed 89.2 ± 28.8 g/day.

Cognitive Performance

Accuracy for the cognitive-performance test and Sternberg tests remained relatively constant from Week 1 to Week 8 for both groups. The ANOVAs yielded no significant results, and no significant changes were seen for any of the tests for either group.

For the cognitive-performance test and all of the Sternberg tests, the reaction-time week main-effect test was significant, reflecting a decrease (i.e., improvement) from Week 1 to Week 8 for both groups. The greatest improvement occurred on the most difficult (Sternberg 6) test, with reaction time decreasing from baseline by 10.7% and 15.0% for the WPL and placebo groups, respectively. Although participants trained on these cognitive tests for 1 hr approximately 48 hr before pre- and posttesting and were not exposed to the tests during the 8 weeks of intervention, these improvements suggest that they may not have trained to asymptote before beginning the study and therefore showed improvement with repetition of the tests. Based on the paired t tests, the only statistically significant improvements were seen for the placebo group during the Sternberg 2 and Sternberg 6 tests and for the WPL group during the Sternberg 4 test. However, in no case was there a significant difference between the WPL group change and the placebo group change. In addition, the percentage of participants who showed large (5% or greater) improvement was comparable for the WPL and placebo groups (58% vs. 46% for the cognitive-performance test, 36% vs. 46% for Sternberg 2, 50% vs. 46% for Sternberg 4, and 57% vs. 73% for Sternberg 6), and in no case were they significantly different.

Finally, scores on the Stanford Sleepiness Scale remained essentially unchanged from Week 1 to Week 8 in both groups, with no significant results found. See Table 3.

Discussion

The primary findings of this investigation were that 8 weeks of supplemental whey protein with leucine resulted in mild increases in muscle strength and lean body mass but did not promote increases in endurance performance or cognitive performance. The increases in strength and lean body mass were not as large as was demonstrated in two previous, similar investigations (Cribb, Williams, Stathis, Carey, & Hayes, 2007; Willoughby, Stout, &

Wilborn, 2007) but appear to be greater than in two other previous, similar investigations (Kerksick et al., 2006; Mielke et al., 2009).

Despite a number of investigations there is not yet a clear consensus on the influence of supplemental whey protein and/or leucine on strength as reflected by 1-RM bench press (BP). Kerksick et al. (2006) supplemented participants with whey and casein, whey and BCAAs, or placebo over 10 weeks of RT. They observed a significant increase in 1-RM BP in all 3 groups with no differences between the groups, although the whey and casein group trended slightly higher. Similarly, Mielke et al. (2009) found that a whey and leucine group, a carbohydrate group, and a control group all increased their 1-RM BP significantly over 8 weeks with no differences between groups. In contrast, Cribb et al. (2007) reported that participants supplemented with whey protein over 11 weeks of RT significantly increased their 1-RM BP over their Week 0 baseline and that the change in the wheyprotein group was significantly greater than that of a carbohydrate-supplemented group. Burke et al. (2001) and Willoughby et al. (2007) observed that both a protein and a placebo group experienced significant increases in strength as reflected by 1-RM BP over a 10-week period, with the increases for the protein group being greater than those of the placebo group. Our results lend support, albeit mild, to those of Willoughby et al. and Cribb et al. (2007); we observed a significant 1-RM BP increase of 3.54 kg from Week 1 to Week 8 in the WPL group and a nonsignificant 1.32 kg increase in the carbohydrate group.

One notable difference between the current study and most of those that have observed significant physiological and performance gains is the length of the trials. The current study was 8 weeks long, whereas the studies showing the greatest gains from the use of whey protein and/or leucine were 10 weeks (Burke et al., 2001; Willoughby et al., 2007) or 11 weeks (Cribb et al., 2007) in duration. Another important distinction is that all of the aforementioned studies incorporated a standardized RT program for participants in all groups, whereas the current study did not. This study simply insisted that participants maintain the USAF minimums for physical training, which did not include substantial traditional, external weight-based RT (e.g., bench press, dead lift, etc.). We performed a more detailed retrospective inspection of the bench-press data and found that the percentage of participants who routinely performed low levels of RT for the duration of the study but who showed large (5% or greater) improvement in the bench press were about the same in the WPL and placebo groups (25.0% vs. 16.7%, respectively). However, for participants who performed medium to high levels of RT (using external weights) a higher percentage showed large improvements in the WPL group than in the placebo group (54.4% vs. 25.0%). These numbers, although not statistically significant, suggest that an individual who routinely follows a rigorous RT program may benefit from supplemental whey and leucine to a greater degree than one who does not follow such a program.

We also observed a significant increase in push-ups (5.4) by the WPL group, whereas the placebo group showed a nonsignificant increase of 3.3 push-ups. In the other muscle-endurance parameters we measured (crunches, chin-ups), none of the changes from Week 0 to Week 8 were significant, nor were there observed differences between groups, although the scores of the WPL group did trend slightly higher. Push-ups and crunches are an integral part of the USAF physical-training program, and nearly all participants performed them regularly during the study. Chin-ups are not a standard USAF exercise. Most previous studies that have examined the influence of supplemental protein on physical performance have not examined its influence on muscle endurance. However, Kerksick et al. (2006) reported no significant differences over 10 weeks in number of BP repetitions at 80% 1-RM, with no differences between groups. Similarly, Mielke et al. (2009) did not observe significant differences in the number of BP and legextension repetitions between a whey and leucine group and a control group after 8 weeks of supplementation.

No differences in cardiorespiratory endurance were demonstrated by either group in their 3-mile-run times over the 8-week test period. This is in contrast to Crowe et al. (2006), who observed that rowers supplemented with leucine for 6 weeks improved their 70–75% VO_{2peak} rowing time to exhaustion by over 10 min while a placebo group did not improve. The disparity may be the result of moderate-intensity rowing's potentially placing a greater demand on strength characteristics than moderate-intensity running. In the current study high-intensity running performance was not influenced by supplementation. However, because the 40-yd sprint was done at the end of a 3-mile run it is unlikely that our sprint test was a true test of power.

The WPL group experienced significant increases from Week 0 to Week 8 in total body weight and lean body mass while the placebo group did not. Body composition did not change significantly over time for either group, nor was there a difference between groups. The gain in lean body mass we observed mirrors gains observed in previous studies (Burke et al., 2001; Cribb, Williams, Carey, & Hayes, 2006; Cribb et al., 2007; Kerksick et al., 2006; Willoughby et al., 2007). Koopman et al. (2005, 2006) demonstrated that ingesting supplemental whey protein with leucine significantly increases nitrogen balance. Such an increase over an 8-week period would explain the increase in lean body mass that we observed. The other possibility is that the WPL group simply consumed more calories (and/or more protein beyond the supplement) or expended fewer calories over the 6-week period than did the placebo group. However, the results from 3-day diet logs recorded twice over the 8 weeks do not indicate a difference in calories consumed between groups. Although we instructed our participants to eat their usual diet and took diet-analysis "snapshots," we did not control or track total calories consumed or expended over the entire course of the study, just as we did not control participants' physical-training regimens.

This was based on a goal of being able to tell our airmen that simply adding a daily whey and leucine supplement to their normal routines would (or would not) provide enhanced performance.

We hypothesized that supplemental WPL could enhance cognitive performance during physical fatigue by staving off central fatigue and/or up-regulating mammalian target of rapamycin (mTOR). Central fatigue implicates serotonin accumulation as a primary cause of decreased physical and cognitive performance (Romanowski & Grabiec, 1974). Tryptophan entering the central nervous system increases production of serotonin, potentially producing central fatigue. The mechanism responsible for transporting tryptophan into the central nervous system is the same system that transports BCAAs like leucine into the central nervous system (Chaouloff, 1989). If BCAA concentration and, therefore, the ratio of BCAAs to unbound tryptophan are increased, the BCAAs compete with unbound tryptophan for entrance into the central nervous system. This could lead to less serotonin production, staving off central fatigue and a hypothesized enhancement or maintenance of performance. Up-regulation of mTOR is another mechanism by which whey and leucine supplementation could potentially benefit cognitive performance. mTOR is a complex protein integrating signals of the energetic status of the cell and environmental stimuli to control protein synthesis and breakdown, thereby controlling cell growth. Although research as to the cause and effect of increased mTOR levels is incomplete, it is suspected to positively influence not only strength and lean body mass (Bodine, 2006) but also cognition and learning (Klann & Sweatt, 2006). Leucine supplementation appears to be an up-regulator of mTOR (Norton, 2006). However, despite the theoretical benefits of these two systems, cognitive-test measures in the current protocol were generally unchanged from Week 0 to Week 8 and between groups.

Some protocols have indeed reported a positive effect of BCAA supplementation on cognitive performance compared with water (Blomstrand et al., 1997; Strüder et al., 1998) or carbohydrate ingestion (Hassmén et al., 1994). However, none of these studies reported providing an isocaloric control condition, Portier et al. (2008) did provide an isocaloric control. Their participants ate a "standard" diet or isocaloric BCAA-supplemented diet during a 32-hr sailing competition. Although they did not report differences in physical performance or in other cognitive-performance tests between groups, they did observe that the standard-diet group suffered a significant decrease in short-term memory performance over the event while the BCAA-supplemented group did not. Cheuvront et al. (2004) also provided an isocaloric placebo but failed to observe any influence of BCAA supplementation on cognition in hypohydrated participants before or after a strenuous cycling bout in the heat. Contrary to the current study, none of these studies administered whey protein with leucine. Instead they used various combinations of valine, leucine, and isoleucine. In addition, previous studies examining the effect of BCAA supplementation on cognition used single or short-term doses. The current protocol appears to be the first to examine the effect of chronic amino acid supplementation on cognition after exercise. However, results of this study did not show any evidence of a positive effect of whey-protein and leucine supplementation on cognition. We suspect that the exercise stress our participants experienced may not have been severe enough to engender substantial central fatigue.

Conclusions

Based on the result of this investigation, although there is a lack of strong statistical evidence, we observed sufficient trends to suggest that 8 weeks of WPL supplementation while adhering to standard USAF physical-training guidelines may be mildly effective at increasing lean body mass and upper body muscle strength. Individuals who routinely follow a vigorous RT program may benefit to a greater degree than those who do not follow such a program. However, such a supplementation regimen appears to be ineffective at influencing endurance performance or cognitive performance. As such, we suggest that WPL supplementation may provide some benefit to athletes and military personnel whose specialties depend highly on strength.

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References

- American College of Sports Medicine. (2000). ACSM's guidelines for exercise testing and prescription (6th ed.). Philadelphia, PA: Lippincott Williams & Williams.
- Blomstrand, E., Hassmén, P., Eckblom, B., & Newsholme, E.A. (1991). Administration of branched-chain amino acids during sustained exercise—Effects on performance and on plasma concentration of some amino acids. European Journal of Applied Physiology, 63, 83–88.
- Blomstrand, E.S., Hassmén, P., Eckblom, B., & Newsholme, E.A. (1997). Influence of ingesting a solution of branchedchain amino acids on perceived exertion during exercise. *Acta Physiologica Scandinavica*, 159, 41–49.
- Bodine S.C. (2006). mTOR signaling and the molecular adaptation to resistance exercise. *Medicine and Science in Sports* and Exercise, 38(11), 1950–1957.
- Bovill M.E., Tharion W.J., & Lieberman H.R. (2003). Nutrition knowledge and supplement use among elite U.S. army soldiers. *Military Medicine*, 168(12), 997–1000.
- Burke, D.G., Chilibeck, P.D., Davidson, K.S., Candow, D.G., Farthing, J., & Smith-Palmer, T. (2001). The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *International Journal of Sport Nutrition and Exercise Metabolism*, 11(3), 349–364.

- Chaouloff, F. (1989). Physical exercise and brain monoamines: A review. *Acta Physiologica Scandinavica*, 137, 1–13.
- Chaouloff, F., Elghozi, J.L., Guezennec, Y., & Laude, D. (1985).
 Effects of conditioned running on plasma, liver and brain tryptophan and 5-HT metabolism of rat. *British Journal of Pharmacology*, 86, 33–41.
- Cheuvront S.N., Carter R., Kolka M.A., Lieberman H.R., Kellogg M.D., & Sawka M.N. (2004). Branched-chain amino acid supplementation and human performance when hypohydrated in the heat. *Journal of Applied Physiology*, 97(4), 1275–1282.
- Coburn, J.W., Housh, D.J., Housh, T.J., Malek, M.H., Beck, T.W., Cramer, J.T., . . . Donlin, P.E. (2006). Effects of leucine and whey protein supplementation during eight weeks of unilateral resistance training. *Journal of Strength* and Conditioning Research, 20(2), 284–291.
- Cribb P.J., Williams A.D., Carey M.F., & Hayes A. (2006). The effect of whey isolate and resistance training on strength, body composition, and plasma glutamine. *International Journal of Sport Nutrition and Exercise Metabolism*, 16(5), 494–509.
- Cribb, P.J., Williams, A.D., Stathis, C.G., Carey, M.F., & Hayes, A. (2007). Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. *Medicine and Science in Sports and Exercise*, 39(2), 298–307.
- Crowe M.J., Weatherson J.N., & Bowden B.F. (2006). Effects of dietary leucine supplementation on exercise performance. *European Journal of Applied Physiology*, 97(6), 664–672.
- Greenwood, M.R.C., & Oria, M. (Eds.), Committee on Dietary Supplement Use by Military Personnel, Institute of Medicine. (2008). Use of dietary supplements by military personnel. Washington, DC: The National Academies Press.
- Hassmen, P., Blomstrand, E.S., Eckblom, B., & Newsholme, E.A. (1994). Branched-chain amino acid supplementation during 30-km competitive run: Mood and cognitive performance. *Nutrition (Burbank, Los Angeles County, Calif.)*, 10, 405–410.
- Jones W.P., Loe S.A., Krach S.K. Rager R.Y., & Jones H.M. (2008) Clinical Neuropsychology, 22(2):305–20.
- Kerksick C.M., Rasmussen C.J., Lancaster S.L., Magu B., Smith P., Melto C., . . . Kreider, R.B. (2006). The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. *Journal of Strength & Conditioning Research*, 20(3), 643–653.
- Klann E., Sweatt J.D. (2008). Altered protein synthesis is a trigger for long-term memory formation. *Neurobiology* of Learning and Memory, 89(3), 247–259.
- Koopman R., Verdik L., Manders R.J., Gijsen A.P., Gorselink M., Pijpers E., ... van Loon, L.J. (2006). Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. American Journal of Clinical Nutrition, 84(3), 623–632.
- Koopman R., Wagenmakers A.J., Manders R.J., Zormnce A.H., Senden J.M., Goreslink M., . . . van Loon, L.J. (2005). Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *American Journal of Physiology*. *Endocrinology and Metabolism*, 288(4), 645–653.

- Mielke, M., Housh, T., Malek, M., Beck, T., Schmidt, R., Johnson, G., & Housh, D.J. (2009). The effects of whey protein and leucine supplementation on strength, muscular endurance, and body composition during resistance training. *Journal of Exercise Physiology Online*, 12(5), 39–50.
- Norton, L.E., & Layman, D.K. (2006). Leucine regulates translation of protein synthesis in skeletal muscle after exercise. *Journal of Nutrition*, 136(2), 533S–537S.
- Portier H., Chatard J.C., Filaire E., Jaunet-Devienne M.F., Robert A., & Guezennec C.Y. (2008). Effects of branchedchain amino acids supplementation on physiological and psychological performance during an offshore sailing race. *European Journal of Applied Physiology*, 104(5), 787–794.
- Romanowski, W., & Grabiec, S. (1974). The role of serotonin in the mechanism of central fatigue. Acta Physiologica Polonica, 25, 127–134.
- Strüder H.K., Hollmann W., Platen P., Donike M., Gotzmann A., & Weber K. (1998). Influence of paroxetine, branched-chain amino acids and tyrosine on neuroendocrine system responses and fatigue in humans. Hormone and Metabolic Research, 30(4), 188–194.
- Willoughby, D.S., Stout, J.R., & Wilborn, C.D. (2007). Effects of resistance training and protein plus amino acid supplementation on muscle anabolism, mass, and strength. *Amino Acids*, 32(4), 467–477.